Probable Identification of γ-, TS-, R- and S-Caseins as Fragments of β-Casein

Abstract

From the results of amino acid analysis, molecular weight determinations, peptide mapping, and end-group analysis, it was believed that bovine γ-, TS-, R- and S-caseins might be pieces of different sizes of the β-casein molecule. Determination of the sequence of the first 10 to 15 amino acid residues at the N-terminal part of these proteins by a sequencer lends credence to the hypothesis because the sequences fit, at appropriate locations, the partial amino acid sequence of β-casein worked out by Ribadeau Dumas, Grosclaude and Mercier.

Discussion of Literature

The classical γ-fraction of bovine milk casein has been shown to be a mixture of proteins. It contains not only a protein properly called γ-casein but other proteins as well. Some of these have been purified and characterized and have been designated TS- (temperature-sensitive), R- and S-caseins. Polymorphism in these minor components of micellar casein has been demonstrated; their biosynthesis, like that of the major components of bovine casein, α_{s1}-, β- and κ-caseins, is genetically controlled. Typing of caseins in milks from individual cows disclosed, furthermore, interrelationships in the occurrence of polymorphs of β-, γ-, TS-, R- and S-caseins. For example, the milk of a cow typed as containing β-casein B always had the same polymorph of γ-casein (designated B), a TS-casein polymorph (designated B) and S-casein; similarly, β-casein A^2, γ-A^2, TS-A^2 and R-caseins always were found together (1, 8).

Evidence from amino acid analysis of β-caseins A^2 and B and of γ-caseins A^2 and B strongly supported a relationship in the biosynthesis of these caseins, for the same differences in amino acid composition, presumably two substitutions (Ser → Arg and Pro → His) which distinguish the β-casein polymorphs, are found also in the corresponding γ-caseins (2). From information then available, it was thought that γ-casein was a larger molecule than β-casein. We believe now that γ-casein is the smaller molecule and that, in fact, it represents the C-terminal portion of some 180 amino acid residues of β-casein, which consists of 208 to 209 residues and has a molecular weight of at least 24,000 (2, 7). We find, also, that the TS-, R- and S-caseins are even smaller molecules of about 100 residues each and propose that these represent essentially the C-terminal half common to γ- and β-caseins.

The evidence, as yet unpublished, for the hypothesis that γ-, TS-, R- and S-caseins are fragments of β-casein, may be summarized as follows. Recent comparative measurements of molecular weight by sedimentation equilibrium in aqueous solutions at low temperature and in 6 M guanidine · HCl, and by dodecyl sulfate-polyacrylamide gel electrophoresis showed β-casein to be the largest molecule, γ-casein somewhat smaller with a molecular weight of about 21,000, and the TS-, R- and S-caseins the smallest at 12,000. Amino acid analysis of the TS-, R- and S-casein polymorphs revealed a close similarity in composition. A single substitution, Ser→Arg, would account for the difference in composition between TS-A^2 and S, as well as for the difference between R and TS-B, and the pair TS-A^2 and S were larger than the pair R and TS-B only by two amino acid residues, Lys and His. Relatedness in amino acid composition among all of the proteins under discussion was apparent and peptide maps of chymotryptic digests indicated many similarities in primary structure. The N-terminal residue of the β-caseins was Arg, of the γ-caseins, Lys, of TS-A^2 and S, His, and of R and TS-B, Glu. The identical sequence, -Ile-Ile-Val · OH was found to be C-terminal in all.

In 1970 Ribadeau Dumas et al. (5, 6) described the isolation and amino acid composition of peptides following cleavage of β-casein A^2 with trypsin and with cyanogen bromide. It occurred to us to compare results of our amino acid analysis of γ-A^2, TS-A^2 and R-caseins with the amino acid composition of the β-casein peptides. If, from the total amino acids of β-casein A^2, one subtracts the amino acids in tryptic peptides T1 + T9 (5), the remainder is exactly equal to the amino acid composition of γ-casein A^2. Also, if one subtracts from β-casein A^2, the amino acids in cyanogen bromide peptides CN1 + CN5 + CN6 (5), there remains R-casein, from which 1 Glu and 1 Met are missing. Since peptide T1 was considered to be the phosphopeptide known to constitute the N-terminal part of β-casein, and since peptide CN1 was a larger peptide con-

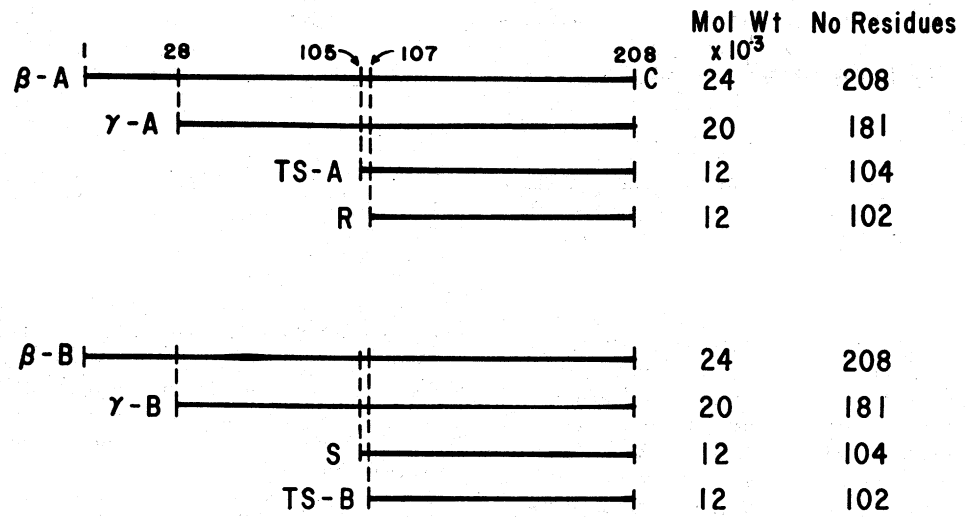

FIG. 1 Diagram of the peptide chain of β-casein A^2 according to Ribadeau Dumas et al. (7) with Residues 28, 105, and 107 indicating proposed locations of N-terminal amino acids of γ-A^2, TS-A^2 and R-caseins and a similar scheme for the β-B family.

taining T1, it seemed possible that our smaller proteins could represent pieces of β-casein toward the C-terminus, and perhaps even probable because of the identity of the C-terminal tripeptide sequence in all the proteins.

Some months later Ribadeau Dumas et al. (7), in another paper on the primary structure of β-casein A^2, reported the ordering of all of the tryptic and "CNBr" peptides. At the N-terminus the order was H · T1-T9- or H · CN1-CN5-CN6-. Furthermore, in the published partial sequence the presence of Lys at Position 28 strengthened the argument that γ-casein begins at this point, and the location of a His at 105 and a Glx at 107 favored the idea that these were the N-termini of TS-A^2 and R-caseins. Our hypothesis, which rested on a similar order deduced from the evidence previously summarized, now appeared much more likely.

Experimental Procedures

Sequence determinations on the N-terminal portions of γ-A^3, TS-A^2 and TS-B caseins[1] were made, with the cooperation of Dr. P. W. D. Mitchell, on the sequencer at The Franklin Institute in Philadelphia. Derivatized residues were identified by gas or thin layer chromatography or, following hydrolysis, with an amino acid analyzer, or by a combination of these methods. Question marks are included where identification is uncertain.

Results

The N-terminal sequence for the first 16 residues of γ-casein A^3 was: H · Lys-Ile-Glu-Lys-Phe-Gln-Ser?-Glu-Glu-Gln-Gln-Glu-Glx-Gln-Asx-Gln-. Ribadeau Dumas et al. (7) reported the sequence of Residues 28 to 46 in β-casein A^2 as -Lys-(Ile, Glx)-Lys-(Asx_2, Thr, Ser, Glx_9, Leu, Phe)-. The two sequences are completely compatible and perhaps identical.

The N-terminal sequence of the first 12 residues of TS-A^2 casein was H·His-Lys-Glx-Met-Pro-Phe-Pro-Lys-Tyr-Pro-Val-Glu-. The reported sequence of Residues 105 to 142 in β-casein A^2 is -His-Lys-Glx-Met-(Pro_2, Phe)-Lys(Asx_2, Thr_3, Ser_3, Glx_5, Pro_4, Val_2, Leu_7, Tyr, Phe, Trp, His)-.

The N-terminal sequence of the first 10 residues of TS-B casein was H·Glx-Met-Pro-Phe-Pro-Lys-Tyr-Pro?-Val-Glx?-, again in excellent agreement with the reported sequence for Residues 107 to 142.

A schematic drawing, Figure 1, illustrates the proposed positioning of the sequences of the smaller molecules within the sequence of β-casein reported by Ribadeau Dumas et al. (7)

[1] Because of the limited supply of several of the protein polymorphs, sequence determinations were made on γ-A^3 rather than γ-A^2 casein, and on TS-B rather than R-casein; γ-A^3 differs from γ-A^2 only by a Gln → His substitution at Position 105, and TS-B from R in an Arg → Ser substitution, location unknown. Neither difference affects the arguments presented.

We conclude from the evidence that in all probability γ-, TS-, R- and S-caseins are pieces of the β-casein molecule. One uncertainty should be mentioned. It has been thought that all the phosphorus in β-casein occurs in the N-terminal phosphopeptide, T1, Residues 1 to 25. However, γ-casein, which begins at Position 28 in the sequence of β-casein, is known to contain one P per molecule. The location of this atom of phosphorus, presumably as a phosphorylated amino acid, remains to be established.

Our conclusion, if substantiated, may have important implications for more understanding of the biosynthesis of casein micelles, in particular, and of proteins in general.

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